

REMARKS

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and the reasons that follow. As the following paragraphs explain, Applicants believe that each of the objections and the rejections is overcome, placing the case in condition for allowance. Accordingly, an early indication of allowability is respectfully requested.

Applicants have added claims 20-25. The application as filed amply supports these new claims, as evidenced, for example, by Examples 5 and 29 and original claims 1-6. Following entry of these revisions, claims 1, 2, 4, 6, 8 and 10-25 will be pending. Claims 3, 5, 7 and 9 are canceled. Claims 8 and 10-19 are withdrawn. Applicants reserve the right to file a divisional or continuing application as a vehicle for the subject matter of any withdrawn or canceled claims.

Claim Objections

In section 6 of the Office Action, the Examiner has objected claims 3-7 and 9 for improper multiple dependency. The claims in question have been revised or replaced by new claims 20-25. Accordingly, Applicants believe that the basis for these objections have been obviated.

Rejections Under 35 U.S.C. § 112

In sections 7-9 of the Office Action, the Examiner has rejected claims 5 and 6 for indefiniteness. Claim 5 has been canceled. Claim 6 has been revised to recite "X-linked hypophosphatemic rickets," pursuant to the Examiner's suggestion.

In sections 10-24 of the Office Action, the Examiner has rejected claims 1-7 and 9 for alleged lack of enablement and alleged insufficient written description. Claims 3, 5, 7 and 9 have been canceled, and the Examiner's stated rationale is inapposite to revised claims 1 and 6, as well as to new claims 20-25.

BEST AVAILABLE COPY

Rejections Under 35 U.S.C. § 102

(I) In sections 25-35 of the Office Action, the Examiner has rejected claims 1, 2, 4-7 and 9 for anticipation by U.S. Publication 2002/0156001 and by international application WO 01/61007, respectively. Claims 5, 7 and 9 have been canceled.

As claim 1 prescribes, the present invention relates to an antibody that recognizes the sequence of amino acids that is found between the 180th and 194th and between the 237th and 251st amino acid residues of SEQ ID NO: 1, respectively. The Examiner contends that the antibodies taught by each of the '001 publication and the '007 publication "would have the ability to bind to [these] amino acid sequences ...and treat the same diseases" (page 9, lines 1-3 and page 10, lines 7-9, respectively).

This allegation runs contrary, however, to paragraph [0307] of the '001 publication, which explicitly teaches rabbit anti-human FGF23 polyclonal antibodies that were raised against the peptide CSQELPSAEDNSPMASD-COOH, which corresponds to residues 206-222 of the human FGF23. It is apparent, therefore, that the reference in no way implicates an ability to bind to the amino acid sequence between residues 180 and 194 or between residues 237 and 251 of SEQ ID NO: 1, as recited in claim 1. In effect, the antibody of the present invention binds an epitope that differs taught for the antibody of the publication, and so it is not surprising that these antibodies may not have the same structure or function.

Along the same line, publication '007 teaches that antibodies may be obtained in an animal by immunization with purified FGF23 protein or with FGF23 peptides. Nevertheless, it does not disclose an anti-FGF23 antibody raised against the presently recited sequences. Furthermore, it does not even hint at using an antibody to treat X-linked hypophosphatemic rickets, hypophosphatemia, osteoporosis or renal failure, as claim 6 prescribes.

It is apparent, therefore, that neither reference discloses each and every aspect of Applicants' claimed invention. Accordingly, no cogent basis exists for rejecting Claim 1 and its dependents for anticipation by the cited publications, and the rejections in question consequently should be withdrawn.

(II) In sections 36-39 of the Office Action, the Examiner has rejected claim 1 for anticipation by Lorenz-Depierux *et al.*

The Examiner correctly points out that Lorenz-Depierux discloses an antibody produced against a peptide with a sequence corresponding to residues 229-243 of human FGF23. The antibody of claim 1, however, recognizes the amino acid sequence between residues 180 and 194 or between residues 237 and 251. There is a seven-residue overlap of the epitopes recognized by the antibody of the publication and the claimed antibody.

To this end, Applicants submit Exhibit A, which is a copy of the pages 58 to 62 of the *ENCYCLOPEDIA OF MOLECULAR BIOLOGY* (1994). As Exhibit A indicates, when an exogenous protein antigen is injected to an animal to produce an antibody, the antigen is processed in the cell and the resultant peptide of the antigen binds to class II MHC molecule on the surface of the cell (page 59, left column, last two paragraphs, same page, right column, first paragraph and page 60, Fig. A43). The peptide antigen binding to class II MHC molecule usually consists of 13-25 amino acids (page 59, right column, first sentence of the last paragraph). At the time when the present invention was made, in other words, the expectation in the field was that a site recognized by an antibody is determined by 13-25 amino acids.

With this background, the skilled person informed by the cited art would have understood that the 7-residue overlap between the 15 residue-long epitopes recognized by the Lorenz-Depierux's antibody and by the claimed antibody, respectively, does not mean that their epitopes are the same. In particular, the resultant antibodies might well have dissimilar structures and/or function, contrary to what the Examiner has asserted.

Accordingly, it is not true that the cited reference effectively conveyed the claimed invention to the knowledgeable public prior to the filing date of claim 1. For this reason there can be no anticipation of claim 1, and the rejection at issue should be withdrawn.

(III) In sections 40-44 of the Office Action, the Examiner has rejected claims 1, 2, 4-7 and 9 for anticipation by U.S. Publication 2004/0082506.

In the present context, the prior art date of a reference under 102(e)(1) may be the international filing date, if (i) the corresponding international filing date is on or after November 29, 2000, (ii) the corresponding international application designated the US, **and** (iii) the corresponding international application was published under the PCT Article 21(2) in the English language. The cited '506 publication was a national phase entry of international application PCT/JP01/06944, which was published in **Japanese** on February 21, 2002, as WO 02/14504 A1.

Accordingly, the 102(e)-effective date of the reference is February 21, 2002. On the other hand, the present application claims priority to two Japanese applications, one of which, No. 2001-401689, was filed on December 28, 2001. The present claims are fully supported by the '689 priority application, as evidenced by each of the certified translation of Japanese patent application Nos. 2001-401689 and 2002-262020 (submitted herewith). See, e.g., the certified translation of 2001-401689 at page 7, third full paragraph. Therefore, the effective filing date of the present claims is before the art-effective date of the cited '506 publication, warranting withdrawal of this Section 102(e) rejection.

Double-Patenting Rejection

In section 45-53 of the Office Action, the Examiner has rejected claims 1, 2, 4-7, and 9 provisionally, for obviousness-type double patenting over claims 10-19 of U.S. Application 10/344,339. As Example 10 of the '339 application indicates, however, the cited claims relate to antibodies raised against peptides consisting of 20 amino acids starting from residue 48 or from residue 114. Conversely, the cited claims do not disclose an antibody that recognizes the amino acid sequence between the 180th and 194th or between the 237th and 251st amino acid of SEQ ID NO: 1, as claim 1 recites.

Moreover, Applicants have discovered these sequences are relevant to metabolites resulting from the cleavage of FGF23 *in situ*. Thus, cleavage occurs between residues 179 and 180, as well as between residues 196 and 197, as described in paragraphs [0039] and [0055] of the present application. Accordingly, an antibody raised against either of these sequences, as claimed, can be expected to recognize physiological metabolites of FGF23 in

serum, a property that is wholly unexpected in view of the prior art of record, including the '339 application, which says nothing of such metabolites.

In light of the foregoing, therefore, claims 1, 2, 4-7, and 9 are not rendered obvious by the cited claims of the '339 application. The double-patenting rejection therefore should be withdrawn.


CONCLUSION

In view of amendments and remarks presented above, Applicants respectfully submit that all of the pending claims are now in condition for allowance. An early notice to this effect is earnestly solicited. If there are any questions regarding the application, the Examiner is invited to contact the undersigned at the number below.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 CFR §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check or credit card payment form being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicants hereby petition for such extension under 37 CFR §1.136 and authorize payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

Date 12 July 2006
FOLEY & LARDNER LLP
Customer Number: 22428
Telephone: (202) 672-5404
Facsimile: (202) 672-5399

By 
Stephen A. Bent
Attorney for Applicants
Registration No. 29,768

THE ENCYCLOPEDIA OF Molecular Biology

EDITOR IN CHIEF
SIR JOHN KENDREW

EXECUTIVE EDITOR
ELEANOR LAWRENCE

b

Blackwell
Science

1994 by
Blackwell Science Ltd
Editorial Offices:
Osney Mead, Oxford OX2 0EL
John Street, London WC1N 2BL
Ainslie Place, Edinburgh EH3 6AJ
8 Main Street, Cambridge,
Massachusetts 02142, USA
University Street, Carlton,
Victoria 3053, Australia

Other Editorial Offices:
Blackwell Science SA
1, rue de Lille, 75007 Paris
France

Blackwell Wissenschafts-Verlag GmbH
Kurfürstendamm 57
10707 Berlin, Germany

Feldgasse 13, A-1238 Wien
Austria

All rights reserved. No part of this
publication may be reproduced, stored
in a retrieval system, or transmitted,
in any form or by any means, electronic,
mechanical, photocopying, recording
or otherwise, except as permitted by the
Copyright, Designs and Patents Act
1988, without the prior permission of
the copyright owner.

First published 1994
Reprinted as a paperback 1995

Printed by Semantic Graphics, Singapore
Printed in Great Britain at the Alden Press Limited,
Oxford and Northampton
Bound by Hartnolls Ltd, Bodmin, Cornwall

DISTRIBUTORS

Marston Book Services Ltd
PO Box 87
Oxford OX2 0DT
(Orders: Tel: 01865 791155
Fax: 01865 791927
Telex: 837515)

USA

Blackwell Science, Inc.
238 Main Street
Cambridge, MA 02142
(Orders: Tel: 800 215-1000
617 876-7000
Fax: 617 492-5263)

Canada

Oxford University Press
70 Wynford Drive
Don Mills
Ontario M3C 1J9
(Orders: Tel: 416 441-2941)

Australia

Blackwell Science Pty Ltd
54 University Street
Carlton, Victoria 3053
(Orders: Tel: 03 347-0300
Fax: 03 349-3016)

A catalogue record for this title
is available from both the British Library
and the Library of Congress

ISBN 0-632-02182-9
0-86542-621-X (pbk)

Codon-anticodon recognition is by complementary BASE PAIRING but can involve some nonstandard base pairing between the base in the first position of the anticodon and the base in the third position of the anticodon (see WOBBLE). Some tRNAs contain modified bases in their anticodon which influence the specificity of codon-anticodon recognition. See: PROTEIN SYNTHESIS.

antidepressant drugs Drugs used in the treatment of depression (see AFFECTIVE DISORDERS). The first effective antidepressants were inhibitors of MONOAMINE OXIDASE (MAO). However the most widely used have been the 'tricyclic' antidepressants, named after the chemical structure of the original members of this group. These drugs block neuronal re-uptake of NORADRENALINE, and in some cases also 5-HYDROXYTRYPTAMINE (5HT), thereby diminishing the rate of removal of neurotransmitter from the synapse (see SYNAPTIC TRANSMISSION). However other actions, such as blockade of α_2 -ADRENERGIC RECEPTORS, have been suggested to be important for some of these compounds. More recently introduced antidepressants include reversible inhibitors of a subtype of MAO, selective inhibitors of 5HT neuronal re-uptake and antagonists of various 5HT RECEPTORS. Tryptophan too has an antidepressant action, presumably by increasing the synthesis of 5HT.

antidiuretic hormone (ADH) VASOPRESSIN.

antigen Any substance or material that is specifically recognized by ANTIBODY or a T CELL RECEPTOR. A distinction may be made between an antigen and an IMMUNOGEN; the latter is a substance or material that induces a specific immune response whereas an antigen may be a structurally related substance or material that is recognized through a cross reaction. In practice the terms are often used synonymously. Secreted antibodies and their corresponding membrane-bound forms can recognize a wide variety of substances as antigens whereas T cell receptors can only recognize fragments of proteins complexed with MHC MOLECULES on cell surfaces. Antigens recognized by immunoglobulin receptors on B cells are subdivided into three categories: T cell-dependent antigens; type 1 T cell-independent antigens; and type 2 T cell-independent antigens (see B CELL DEVELOPMENT). See also: EPITOPE; HAPTEN.

antigen-binding site Site on antibody molecule which recognizes and complexes with specific antigen. Also known as the antibody combining site. See: ANTIBODIES; IMMUNOGLOBULIN STRUCTURE.

antigen presentation The formation of a noncovalent interaction between foreign antigen and MHC class I or class II molecules at the surface of a cell, which allows recognition of foreign antigen by MHC-restricted T CELLS and the generation of an antigen-specific immune response (see MAJOR HISTOCOMPATIBILITY COMPLEX; T CELL RECEPTOR). The term refers to the interaction of foreign antigen with class II MHC molecules on specialized ANTIGEN-PRESENTING CELLS such as macrophages, or describes the association of viral peptides (or other foreign antigens generated intracellularly) with class I MHC molecules on virus-infected cells. Formation of antigen-MHC complexes requires that large

protein antigens be enzymatically processed within the antigen-presenting cell and re-expressed at the cell surface as smaller peptide fragments that then associate with class I or II MHC molecules. Peptide fragments bind to MHC molecules in a groove in the surface. Although each MHC molecule seems to be able to bind a great variety of different peptide fragments, allelic polymorphism in MHC class I and II protein sequences influences the affinity of a particular antigen fragment for a given MHC molecule, and thus also the ability of a particular combination of foreign antigen and MHC to elicit a T cell response. See: ANTIGEN PROCESSING AND PRESENTATION.

antigen-presenting cell (APC) Any of a number of different cell types that can process foreign protein antigens and express them as peptide fragments complexed with class II MHC molecules at the cell surface (see MAJOR HISTOCOMPATIBILITY COMPLEX) and are thus capable of activating antigen-specific T cells and generating an immune response (see T CELL ACTIVATION; T CELL RECEPTOR). Most 'professional' APCs are of the macrophage/monocyte lineage, but B cells, thymocytes and other specialized cells such as dendritic cells (dendrocytes) also express class II MHC molecules and act as antigen-presenting cells (see T CELL ACTIVATION; T CELL DEVELOPMENT). Whereas these cells express class II molecules constitutively, nearly all cells can be induced to express high levels of class II MHC molecules in culture by the addition of the LYMPHOKINE γ -interferon, suggesting that a locally high concentration of γ -interferon *in vivo* may permit otherwise class II-negative cells to function as APCs.

Antigen processing and presentation

THE T lymphocytes (T CELLS) of the immune system can only recognize ANTIGEN in the form of short peptides derived (processed) from the native protein antigen. In addition, they only recognize such peptides as antigenic when these are associated at a cell surface with self glycoproteins encoded by the MAJOR HISTOCOMPATIBILITY COMPLEX (MHC). These peptide-MHC complexes lie at the heart of immune, and most likely many autoimmune, responses. Much current research is therefore aimed at understanding the cell biological and biochemical basis of their generation and expression.

Class I and class II MHC molecules

The MHC encodes two main types of cell-surface glycoprotein: the MHC class I and class II molecules. These are highly POLYMORPHIC in mammals. Polymorphism in MHC antigens is medically inconvenient as it leads to difficulties in organ transplantation, but the polymorphism is presumed to have been selected to increase the peptide binding versatility of the population as a whole and thus its overall resistance to pathogens.

Class I MHC molecules are composed of a membrane-spanning subunit (the heavy chain) which is noncovalently associated with β_2 -microglobulin, and is expressed on almost all cell types. The

three-dimensional structure of a class I MHC molecule reveals a cleft in which most polymorphic residues are clustered and which binds peptide. Class II MHC consists of two membrane-spanning polypeptides which form a peptide-binding site very similar to that on class I (see Plate 6d). Normally, class II MHC expression is limited to certain types of so-called 'professional' ANTIGEN-PRESENTING CELL (APC) such as B lymphocytes (B CELLS), macrophages, dendritic cells, and in humans, activated T cells.

The affinity of T CELL RECEPTORS for antigen is thought to be relatively low, so productive interactions between T cells and MHC-expressing APCs also require one of the two co-receptor molecules that T cells may express (i.e. CD4 or CD8) as well as other adhesion and co-stimulatory molecules. T cells expressing CD4 interact with APCs expressing class II MHC whereas T cells expressing CD8 interact with class I.

These T cell subtypes execute different effector functions on ligation of their antigen receptors. CD8 cells are generally cytotoxic and can kill target cells through exocytosis of lytic granules, whereas CD4 cells have a helper/inducer function mediated at least in part by LYMPHOKINE secretion. It therefore makes sense to express class I on all cell types (as all cells can become virus infected) and to limit class II expression to those cell types with the capacity to proliferate and differentiate in response to T cell lymphokines.

Class I and class II MHC molecules present peptides from different cellular compartments

Early studies on antigen presentation revealed a distinct sensitivity of the process to metabolic inhibitors. Presentation on class I MHC molecules was usually sensitive to inhibitors of PROTEIN SYNTHESIS but was insensitive to drugs such as CHLOROQUINE which block proteolysis in LYSOSOMES. In contrast, presentation on class II MHC molecules was sensitive to chloroquine but rather insensitive to protein synthesis inhibitors [1,2]. This suggested that quite distinct mechanisms were responsible for the generation of peptides captured by class I compared with class II molecules.

It was found that class I MHC molecules were able to present peptides derived from proteins present in cytosolic, nuclear, and even mitochondrial compartments, but could not be loaded when the native protein was taken up into the endosome/lysosome vacuolar system [1]. In contrast, class II MHC molecules seemed only able to present peptides derived from proteins normally found within, or which could gain access to, the endosome system [3].

Presentation of cytosolic proteins on class II MHC molecules has subsequently been demonstrated, however (see Fig. A43), although it is not clear at what stage in class II MHC trafficking these peptides are loaded. Also, presentation of exogenous antigens on class I MHC molecules may be a specialized function of certain cell types and can be demonstrated by engineering the osmotic lysis of endosomes and thus delivery of antigen to the cytosol. MHC molecules therefore provide a constant update in peptide form of cellular protein content (on class I) and environmental protein composition (on class II) for screening by the T-cell system.

Given the distinct effector functions of the T cells which recognize class I and class II MHC, this very striking discrimination among the cellular compartments that can contribute to the peptide repertoire of class I and class II makes sense: for example, if class I MHC molecules readily presented endocytosed viral proteins, uninfected cells as well as infected cells might easily become targets for killing by cytotoxic T cells. Similarly, presentation on class II MHC of peptides from circulating pathogens (which are taken up by ENDOCYTOSIS) is optimized by preventing loading of class II MHC with cytosolic peptides.

How class I and class II MHC molecules control which peptides become bound is now reasonably well understood and is outlined below. First, information on the physical characteristics of the peptides bound is briefly summarized.

The 'endpoint' of antigen processing

The peptides bound to class I and class II differ in length and in other respects (see below) which again suggests that different processing mechanisms are involved. Most naturally processed T cell EPITOPES presented on class I molecules are eight or nine amino-acid residues long, the exact length depending on the class I allele. Sequencing of bulk mixtures of peptides eluted from milligram amounts of purified class I molecules revealed a strong preference for certain residues at certain positions, and these have been termed 'anchor residues' [4]. For example, on the mouse K^b class I molecule, which binds nonamers, tyrosine was always found at position 2 and either isoleucine or leucine at position 9. Other *in vitro* studies show that increasing or decreasing the peptide length by even a single residue dramatically increases the dissociation rate of peptide from class I molecules and consequently increases the instability of the complex [5]. Co-crystals of mouse class I MHC molecules and peptide reveal the importance of the peptide N and C termini as well as the anchor residues in making interactions with the class I binding pocket [6]. Significantly, these crystal structures also underline the importance of interactions between the peptide backbone and side chains from the binding pocket; that is, features common to all peptides contribute significantly to stable binding. There is nonetheless a strong selection during the assembly and maturation of class I molecules for peptides of the right length which also carry appropriate anchor residues. Different alleles of class I select or 'restrict' binding to peptides with distinct anchor motifs. The extent to which peptide delivery for class I binding may also be controlled at the level of peptide production, transport into the ENDOPLASMIC RETICULUM (ER), loading within the ER, or at a post-binding trimming step is not known at the time of writing.

In contrast, class II MHC molecules bind longer peptides of variable length (13–25 residues). Unlike class I, a single peptide determinant can be expressed in different versions that share a common core sequence but display 'ragged' N and C termini [7]. This suggests that, unlike the case of class I MHC binding, peptides of different length but with a common core sequence are all stably bound to class II MHC molecules. Presumably, interactions with the peptide N and C termini do not contribute to stable binding as they do for class I-associated peptides. Heterogeneity

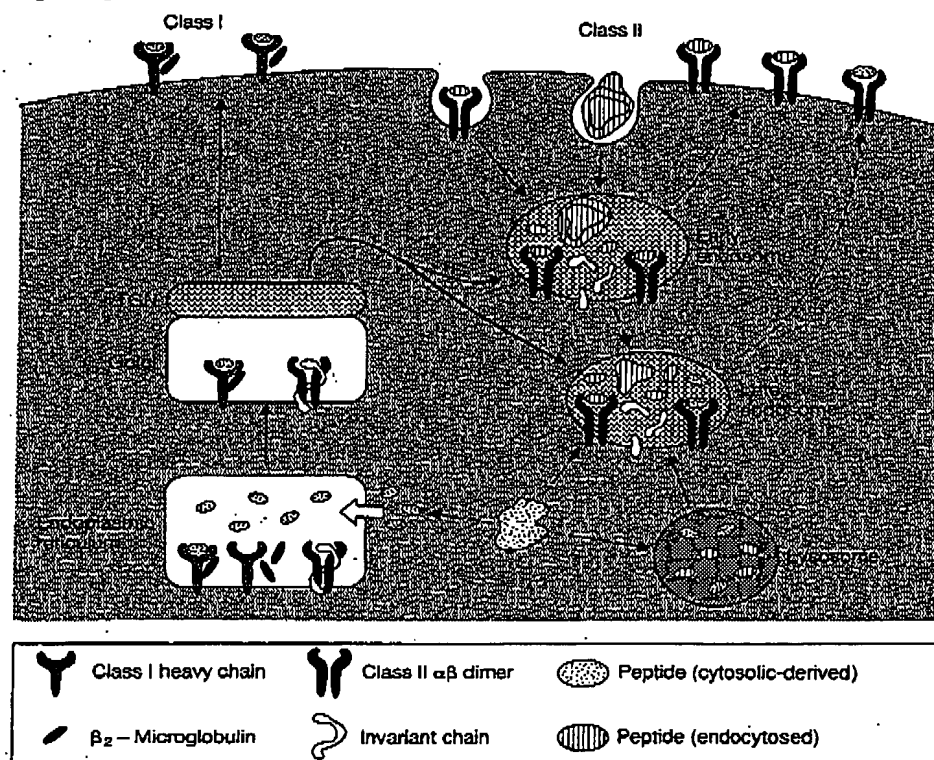


Fig. A43 Schematic representation of processing pathways in an antigen-presenting cell. Protein antigen produced in the cytosolic compartment of a cell (e.g. as a result of virus infection) is processed into peptides which enter the endoplasmic reticulum (ER) (and possibly lysosomes) (dashed arrows indicate pathways not yet established in detail). Class I MHC molecules fold together with β_2 -microglobulin in the secretory pathway, an association which is greatly stabilized by binding by the specific peptides thought to be delivered into the ER by the TAP gene products (see text). The peptide-class I MHC complex is transported through the Golgi apparatus and from there to the cell surface and/or to endosomes. Antigen entering the cell by endocytosis is degraded into peptide fragments in endosomes, where it binds to class II

MHC molecules. Class II molecules are $\alpha\beta$ dimers which assemble in the ER in a complex with the variant chain (Ii) which does not bind peptides. After transport through the Golgi apparatus the $\alpha\beta$ Ii complex is targeted to the endosome/lysosome system where the invariant chain is removed by proteolytic cleavages. Capture of peptides by class II MHC molecules is favoured at the acidic pH of the endosomes. Stable complexes then appear on the cell surface. Neither the compartment in which peptides are loaded onto class II MHC molecules, nor the route that assembled complexes take to the cell surface is certain. Some cytosolic antigens can be presented on class II MHC although it is not clear at which point they feed into this pathway. Adapted from [22].

in peptide length is consistent with the possibility that the peptide-binding groove on class II molecules may be open at both ends, whereas that on class I is closed. Individual peptides bound to class II molecules [8] share some features analogous to the anchor residues identified on class I MHC molecules and these presumably contribute to stable binding.

Abundant peptides recovered from complexes with class II MHC molecules include those from extracellular proteins such as serum albumin, transferrin receptors, and peptides derived from class II MHC molecules themselves. Technical difficulties currently prevent analysis of the numerous peptides present in lower abundance. For class II MHC molecules this includes most T cell epitopes generated following antigen uptake and processing.

Cell biological basis of class I and class II MHC antigen presentation

Why do class I and class II MHC show a strong preference for peptides from endogenous and exogenous antigens respectively?

There is now a satisfactory general explanation although the detailed mechanisms have yet to be unravelled.

1. Class I MHC. A key point to emerge is that peptide binding to class I MHC molecules is obligatory for stable expression of the MHC molecule on the cell surface. Mutant cells in which antigenic peptides cannot be transported from the cytoplasm into the ER fail, in most cases, to express normal levels of surface class I molecules [9]. These cells have mutations in genes mapping within the MHC which are thought to encode 'transporters of antigenic peptides' (TAP genes). The dependence of class I transport on peptide binding can be explained by the finding that at 37°C class I heavy chain does not associate stably with β_2 -microglobulin in the absence of an appropriate peptide. Consequently, the 'quality control' system (see BIP; MOLECULAR CHAPERONES) operating in the secretory pathway prevents such 'empty' molecules from reaching the cell surface (see PROTEIN SECRETION). Those that do are unstable and are quickly degraded [10].

The homology with other peptide transporters in the so-called

ABC (ATP-binding cassette) family (e.g. the CYSTIC FIBROSIS gene product, the multidrug resistance P GLYCOPROTEIN, and the STE6 gene product in yeast), together with evidence that their polymorphism in the rat gives rise to distinct sets of peptides bound to a single class I allele (see below), and the fact that expression of introduced TAP genes in the mutant cells restores surface expression of class I molecules [11], strongly suggests that the TAP proteins transport peptides for loading of class I molecules. Other genes, homologous to subunits of the multicatalytic cytosolic proteinase or 'proteasome' have also been found within the MHC (see PROTEIN DEGRADATION). The precise contribution of these proteins to production and delivery of peptides for loading of class I remains to be firmly established.

Class I molecules therefore arrive on the cell surface fully occupied and moreover are routed there directly from the *trans*-Golgi network [12]. Binding seems to be essentially irreversible, so that even if class I molecules subsequently enter the endocytic pathway they do not usually bind the peptides generated there. This protects cells from sensitization by passively acquired antigen and killing by cytotoxic T cells.

2 Class II MHC. As class II MHC molecules share their early biosynthetic route with class I molecules an explanation is required (1) for the lack of binding of peptides to class II at the stage when class I molecules bind peptides, and (2) for the ability of class II molecules efficiently to capture peptides in the endosome/lysosome system. Both these features of peptide binding seem to be explained by an association of class II molecules with a third polypeptide encoded outside the MHC locus and known as the INVARIANT CHAIN. The invariant chain associates with class II molecule α and β chains shortly after biosynthesis and chaperones the dimer through most of the secretory pathway but does not accumulate to significant levels on the cell surface. Experiments *in vitro* have shown that the invariant chain directly or indirectly prevents peptide binding to class II MHC molecules [13]. Moreover, it is responsible for targeting class II MHC molecules to their eventual site of peptide loading within the endocytic pathway. The targeting signal has been mapped to a short region of the cytosolic domain of the invariant chain [14]. The class II-invariant chain complex is therefore diverted out of the secretory pathway, probably at the *trans*-Golgi network, and delivered to the endosome system. Here, the invariant chain is removed by proteolysis thus generating for the first time a functional binding site for peptides on the $\alpha\beta$ dimer. The acidic pH within the endosome/lysosome system may also optimize peptide binding. As with class I MHC, peptide binding seems to stabilize class II MHC molecule structure to such an extent that a proportion of mature cell-surface class II $\alpha\beta$ dimers remain associated in normally denaturing detergents such as sodium dodecyl sulphate [15].

The essential features of class I versus class II traffic are summarized in Fig. A43 and explain how class I and class II MHC molecules bind peptides present within the secretory and endocytic pathways respectively. As in the case of class I MHC, most cell-surface class II MHC molecules are occupied by peptide and there is some evidence that failure to acquire peptide leads to degradation, in this case in lysosomes [15]. However, cells carrying deletions within the MHC have been described which fail to

present native antigen to T cells but still express cell-surface class II MHC molecules [16]. The class II molecules on these cells are conformationally abnormal but the fact that they appear at all suggests that the coupling of cell surface expression to peptide capture and the attainment of a mature conformation is not as strict for class II as it is for class I molecules. These interesting mutants seem to have lost a crucial but as yet ill-defined function for normal class II maturation.

The overall picture from work on both the structure and the biosynthesis of MHC molecules shows that peptide should be considered an integral subunit of the assembled mature MHC molecule. The immune system has exploited features of the folding and maturation of MHC molecules and cellular trafficking pathways to ensure the display of appropriate peptides to either CD4 or CD8 T cells.

Factors modulating antigen processing and presentation

A central factor limiting antigen presentation on MHC molecules is their biosynthetic output. Antigen presentation on both class I and class II molecules depends on new MHC molecule biosynthesis [3,17]: a cell expressing MHC molecules but not actually making them is not able to present new antigens although it may continue to display the peptides from previously encountered antigens for many days. Unlike other peptide-binding proteins, such as ATP-regulated intracellular molecular chaperones (see MOLECULAR CHAPERONES), MHC molecules in living cells seem to have an essentially monogamous relationship with peptide [5,18]. The biological importance of this persistence of peptides on MHC molecules is illustrated by the behaviour of dendritic cells such as those in the epidermis (Langerhans cells). These cells seem to separate a class II MHC biosynthetic and antigen-processing phase from a later phase in which presentation of pre-existing peptide-MHC antigen complexes persists, but presentation of subsequently offered antigen is ineffective in spite of abundant cell-surface class II molecules. *In vivo* the later phase may correlate with migration of Langerhans cells from sites of antigen capture to sites in lymphoid tissue where antigen is presented to T cells [19].

Other factors modulating the range of peptides presented are poorly characterized at present. Nonetheless, with the basic features now established, modulating influences are of particular interest and importance to immune and autoimmune responses. In the context of class I MHC, a particularly interesting illustration of how the set of peptides presented on the same class I molecule may be modulated comes from work on the rat MHC complex. Here the MHC-linked putative peptide transporter genes show striking polymorphisms which correlate with distinct sets of peptides bound to the same class I molecule. These results provide the best evidence for interaction between the putative peptide transporters and the peptides actually bound, and reveal an unexpected modifying influence on the peptide repertoire expressed on class I molecules [20]. It remains to be seen, for both class I and class II, whether polymorphisms in proteins involved in the peptide generation and loading process are going to be of general importance.

The processing machinery which gives rise to peptides associ-

ated with class II molecules is also poorly characterized as yet and the precise compartmentation of the assembly of class II-peptide complexes is still being worked out. Studies using antigen-specific B lymphocytes reveal that high-affinity ANTIBODIES can affect the course of antigen processing and as a result, its outcome in terms of expressed T cell epitopes [21] and unpublished work). Antibodies may therefore steer the T cell response in a particular direction and so may have an influence on, for example, the effectiveness of vaccines and the dominant representation of certain T cell epitopes in a response.

C. WATTS

- 1 Townsend A. and Bodmer H. (1989) Antigen recognition by class I restricted T cells. *Annu. Rev. Immunol.* 7, 601-624.
- 2 Unanue E.R. (1984) Antigen presenting function of the macrophage. *Annu. Rev. Immunol.* 2, 395-428.
- 3 Morrison, L.A. et al. (1986) Differences in antigen presentation to MHC class I and class II-restricted influenza virus-specific cytolytic T lymphocyte clones. *J. Exp. Med.* 163, 903-921.
- 4 Rotaschke, O. & Falk, K. (1991) Naturally occurring peptide antigens derived from the class I MHC-restricted processing pathway. *Immunol. Today* 12, 447-451.
- 5 Cerundolo, V. et al. (1991) The binding affinity and dissociation rates of peptides for class I MHC molecules. *Eur. J. Immunol.* 21, 2069-2075.
- 6 Matsumura, M. et al. (1992) Emerging principles for the recognition of peptide antigens by MHC class I molecules. *Science* 257, 927-932.
- 7 Hunt, D.F. et al. (1992) Peptides presented to the immune system by the murine class II MHC molecule I-A^b. *Science* 256, 1817-1920.
- 8 Hammer, J. et al. (1992) Identification of a motif for HLA-DR1 binding peptides using M13 display libraries. *J. Exp. Med.* 176, 1007-1013.
- 9 Townsend, A. et al. (1989) Association of class I MHC heavy and light chains induced by viral peptides. *Nature* 340, 443-448.
- 10 Schumaker, T. et al. (1990) Direct binding of peptide empty MHC class I molecules on intact cells and in vitro. *Cell* 62, 563-567.
- 11 Kelly, A. et al. (1992) Assembly and function of the two ABC transporter proteins encoded in the human major histocompatibility complex. *Nature* 355, 641-644.
- 12 Neefjes, J.J. et al. (1990) The biosynthetic pathway of class II but not class I MHC molecules intersects the endocytic pathway. *Cell* 61, 171-183.
- 13 Roche, P. & Cresswell, P. (1990) Invariant chain association with HLA-DR molecules inhibits immunogenic peptide binding. *Nature* 345, 615-618.
- 14 Bakke, O. & Dobberstein B. (1990) MHC class II-associated invariant chain contains a sorting signal for endosomal compartments. *Cell* 63, 707-716.
- 15 Germain, R.N. & Hendrix, L.R. (1991) MHC class II structure, occupancy and surface expression determined by post-endoplasmic reticulum antigen binding. *Nature* 353, 134-139.
- 16 Mellins, E. et al. (1990) Defective processing and presentation of exogenous antigens in mutants with normal HLA class II genes. *Nature* 343, 71-74.
- 17 Davidson, H.W. et al. (1991) Processed antigen binds to newly synthesized MHC Class II molecules in antigen-specific B lymphocytes. *Cell* 67, 105-116.
- 18 Lanzavecchia, A. et al. (1992) Irreversible association of peptides with class II MHC molecules in living cells. *Nature* 357, 249-252.
- 19 Steinman, R.M. (1991) The dendritic cell system and its role in immunogenicity. *Annu. Rev. Immunol.* 9, 271.
- 20 Powis, S. et al. (1992) Effect of polymorphism of an MHC-linked transporter on the peptides assembled in a class I molecule. *Nature* 357, 211-215.
- 21 Davidson, H.W. & Watts, C. (1989) Epitope-directed processing of specific antigen by B lymphocytes. *J. Cell Biol.* 109, 85-92.
- 22 Long, E. (1992) Antigen processing for presentation to CD4⁺ T cells. *New Biologist* 4, 274-282.

antigen receptors See: ANTIBODIES; B CELL DEVELOPMENT; IGM RECEPTOR; IMMUNOGLOBULIN STRUCTURE; T CELL DEVELOPMENT; T CELL RECEPTORS.

antigen recognition The binding of an ANTIGEN by specific receptors on T CELLS and B CELLS of the immune system, which triggers their activation, proliferation, and terminal differentiation to effector cells. See: ANTIBODIES; ANTIGEN PROCESSING AND PRESENTATION; B CELL DEVELOPMENT; IMMUNOGLOBULIN GENES; IMMUNOGLOBULIN STRUCTURE; MAJOR HISTOCOMPATIBILITY COMPLEX; T CELL DEVELOPMENT; T CELL RECEPTORS; T CELL RECEPTOR GENES.

antigenic determinant Molecular configuration on the surface of an antigen that is recognized by an ANTIBODY. See also: EPITOPE.

Antigenic variation in African trypanosomes

AFRICAN trypanosomes are unicellular eukaryotic parasites of mammals transmitted by tsetse flies [1-4]. The trypanosomes *Trypanosoma brucei rhodesiense* and *T. b. gambiense* cause sleeping sickness in humans. Laboratory experiments are mostly done with *T. b. brucei* strains that grow well in rodents (after syringe transmission), but not in humans. In the mammalian host trypanosomes are entirely covered by a dense surface GLYCOPROTEIN coat and ANTIBODIES directed against this coat lyse the trypanosome. By drastically changing the composition of the coat — antigenic variation — a subfraction of the trypanosomes escapes immune lysis, allowing maintenance of a chronic infection and the continued presence of trypanosomes in the bloodstream. A single trypanosome and its progeny can produce more than 100 coats that have no ANTIGENIC DETERMINANTS in common. Coat switching is not induced by antibody and occurs spontaneously at a frequency of 10^{-6} per division in established laboratory strains, but at much higher rates in the field.

The surface coat

The coat of bloodstream trypanosomes [2,5] consists of a single species of protein, the variant specific surface glycoprotein (VSG). VSGs range in size from 400 to 500 amino-acid residues and are anchored in the plasma membrane by a complex glycosylphosphatidylinositol (GPI) tail (see GPI ANCHORS). The three-dimensional structure of two VSGs has been determined at 2.9 Å resolution and the complex GLYCAN part of the tail is completely known. A remarkable feature of the GPI anchor is that its fatty acids are exclusively myristic acid (a saturated C₁₄ fatty acid). This makes the trypanosome vulnerable to myristic acid analogues [6].

When the trypanosome enters the tsetse fly, it shuts off VSG synthesis, sheds its VSG coat and replaces it with a coat of another glycoprotein, known as procyclin or procyclic acidic repetitive protein (PARP). After completing its developmental cycle in the fly salivary gland, the trypanosome regains a VSG coat.

Unusual features of gene expression

Each messenger RNA in trypanosomes starts with the same

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

☒ **BLACK BORDERS**

☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**

☐ **FADED TEXT OR DRAWING**

☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**

☐ **SKEWED/SLANTED IMAGES**

☒ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**

☐ **GRAY SCALE DOCUMENTS**

☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**

☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**

☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.